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14. ABSTRACT The research program aimed to develop self-immolative polymers (SIPs) that respond to thermal or mechanical energy by transforming from covalent polymers into small molecule components with controlled kinetic profiles. Trigger moieties that undergo thermally or mechanochemically driven reactions capable of initiating depolymerization of SIPs are needed for these capabilities. We hypothesized that oxazine-based moieties can serve as a common motif for entryway into this area. These triggering mechanisms each potentiate passive (e.g., spontaneous environmental) and on-demand activation of the SIP. This work is an important step toward					
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## Report Title

Final Report: Development of Thermally- and Mechanically-Triggered Self-Immolative Polymers

### ABSTRACT

The research program aimed to develop self-immolative polymers (SIPs) that respond to thermal or mechanical energy by transforming from covalent polymers into small molecule components with controlled kinetic profiles. Trigger moieties that undergo thermally or mechanochemically driven reactions capable of initiating depolymerization of SIPs are needed for these capabilities. We hypothesized that oxazine-based moieties can serve as a common motif for entryway into this area. These triggering mechanisms each potentiate passive (e.g., spontaneous environmental) and on-demand activation of the SIP. This work is an important step toward understanding structure-reactivity relationships for thermal and mechanochemical SIP triggers.

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**Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:**

**(a) Papers published in peer-reviewed journals (N/A for none)**

<u>Received</u>	<u>Paper</u>
07/23/2015 1.00	Gregory I. Peterson, Derek C. Church, Neal A. Yakelis, Andrew J. Boydston. 1,2-oxazine linker as a thermal trigger for self-immolative polymers, Polymer, (11 2014): 5980. doi: 10.1016/j.polymer.2014.09.048
<b>TOTAL:</b>	<b>1</b>

**Number of Papers published in peer-reviewed journals:**

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**(b) Papers published in non-peer-reviewed journals (N/A for none)**

<u>Received</u>	<u>Paper</u>
<b>TOTAL:</b>	

**Number of Papers published in non peer-reviewed journals:**

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**(c) Presentations**

3,6-Dihydro-1,2-Oxazines for the Thermal Activation of Functional Polymers. Polymers Gordon Research Conference and Graduate Symposium, June 2015. Derek C. Church and Andrew J. Boydston

Number of Presentations: 1.00

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**Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

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**Peer-Reviewed Conference Proceeding publications (other than abstracts):**

Received      Paper

**TOTAL:**

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

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**(d) Manuscripts**

Received      Paper

**TOTAL:**

Number of Manuscripts:

Books

Received      Book

TOTAL:

Received      Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Cottrell Scholar Award and NSF CAREER Award (AJB)

Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	Discipline
Gregory I. Peterson	0.00	
Derek C. Church	0.00	
Michael B. Larsen	0.50	
<b>FTE Equivalent:</b>	<b>0.50</b>	
<b>Total Number:</b>	<b>3</b>	

### Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
Yosuke Ashikari	1.00
<b>FTE Equivalent:</b>	<b>1.00</b>
<b>Total Number:</b>	<b>1</b>

### Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	National Academy Member
Andrew Boydston	0.00	No
Neal Yakelis	0.00	
<b>FTE Equivalent:</b>	<b>0.00</b>	
<b>Total Number:</b>	<b>2</b>	

### Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

### Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: ..... 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense ..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

### Names of Personnel receiving masters degrees

<u>NAME</u>
<b>Total Number:</b>

### Names of personnel receiving PHDs

<u>NAME</u>
Gregory I. Peterson
<b>Total Number:</b>

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**Names of other research staff**

NAME

PERCENT SUPPORTED

**FTE Equivalent:**

**Total Number:**

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**Sub Contractors (DD882)**

**Inventions (DD882)**

**Scientific Progress**

**Technology Transfer**

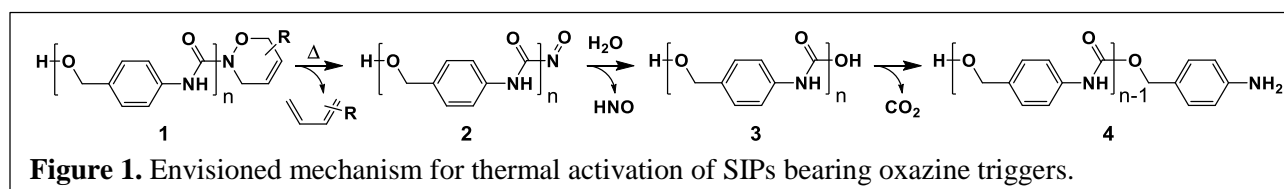
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### Statement of the Problem Studied:

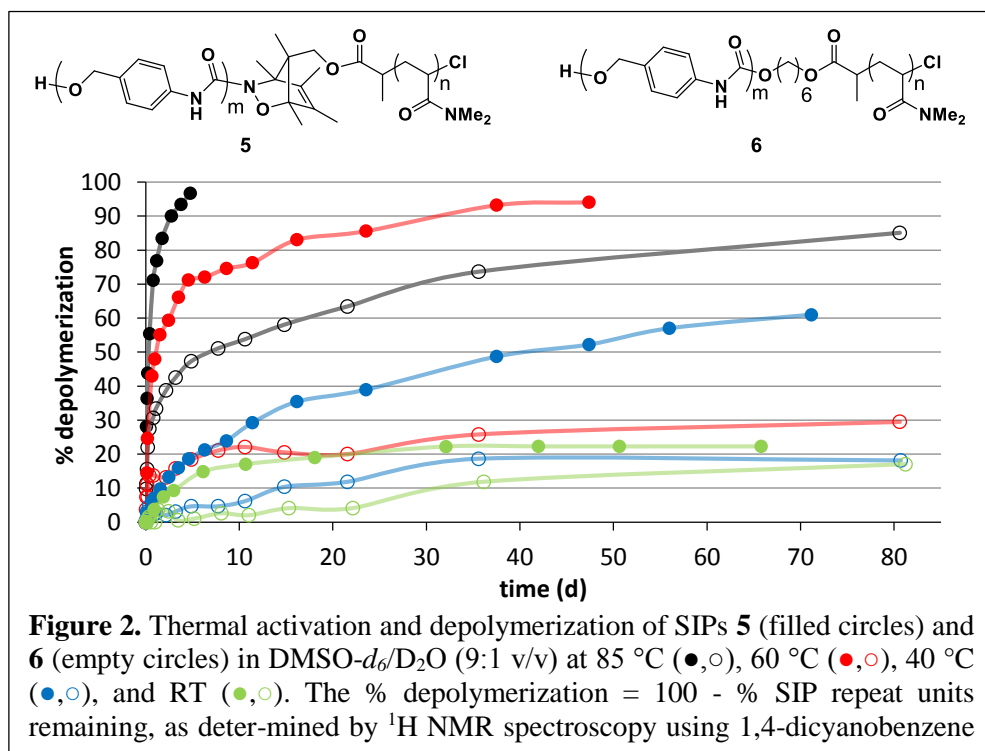
The proposed research aimed to develop self-immolative polymers (SIPs) that respond to thermal or mechanical energy by transforming from covalent polymers into small molecule components with controlled kinetic profiles. Trigger moieties that undergo thermally or mechanochemically driven reactions capable of initiating depolymerization of SIPs are needed for these capabilities. We hypothesized that oxazine-based moieties can serve as a common motif for entryway into this area. These triggering mechanisms each potentiate passive (e.g., spontaneous environmental) and on-demand activation of the SIP. This work is an important step toward understanding structure-reactivity relationships for thermal and mechanochemical SIP triggers.

### Summary of Most Important Research Results:

We hypothesized that thermally-driven cycloreversion of 1,2-oxazines could provide the desired reactivity for triggering SIPs (Figure 1). Specifically, thermal cycloreversion of the oxazine moiety in **1** was expected to liberate carbamoylnitroso species **2**, which would be subject to rapid hydrolysis to give **3**. Decarboxylation would ultimately give the activated SIP **4**. We considered polyurethane-based SIPs derived from *p*-aminobenzyl alcohol to be an attractive and versatile platform since they can be readily adapted for small-molecule release from side chain functionalities, turn-on fluorescence reporting, and solubility in water.



We were able to install the oxazine unit at the junction of an AB diblock copolymer (**5**), achieving an overall A-trigger-B architecture. We also prepared a control system (**6**) absent the oxazine trigger to assess the relative contributions of hydrolysis to the overall depolymerization (Figure 2). To avoid complications arising from insolubility of the SIP block after detachment from the PDMA, we conducted thermal triggering and depolymerization experiments in DMSO-*d*<sub>6</sub>/D<sub>2</sub>O mixtures (9:1 v/v). Figure 2 shows the amount of SIP depolymerization over time as **5** and **6** were each heated at various temperatures. At RT, we observed gradual activation leading to 22% depolymerization after 65 d. As expected, at 40 °C more rapid activation and SIP depolymerization was



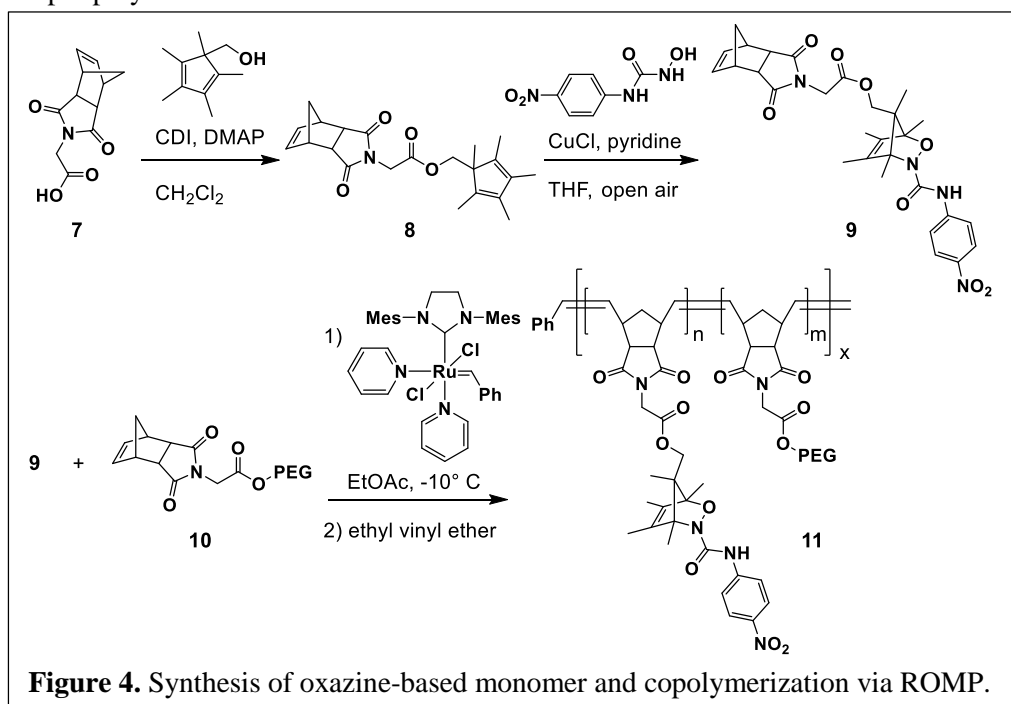


observed, reaching 61% depolymerization after ca. 71 d. At 60 and 85 °C, nearly full consumption of the SIP block was observed after ca. 47 and 5 d, respectively. In contrast, control polymer **6** displayed much lower extents (17 – 18%) of depolymerization when heated at 40 °C for 81 d. At 60 and 85 °C, the rate of depolymerization was clearly much less for **6** than for the oxazine trigger variant (**5**), consistent with different mechanisms of activation between the two. The results from the control polymer suggested to us that hydrolysis does not play a fairly significant role in the degradation of the SIP below 60 °C.

We have also investigated systems in which the oxazine moiety was installed as a side chain functional group (Figure 3), which is expected to enable detachment of SIP units from graft polymers. Additionally, the increased oxazine content of this design provides greater amounts of HNO release, which is attractive for potential therapeutic applications. Our initial approach was to monitor the release of *p*-nitroaniline as a model for the release of SIP head units or therapeutics that are based upon amines.

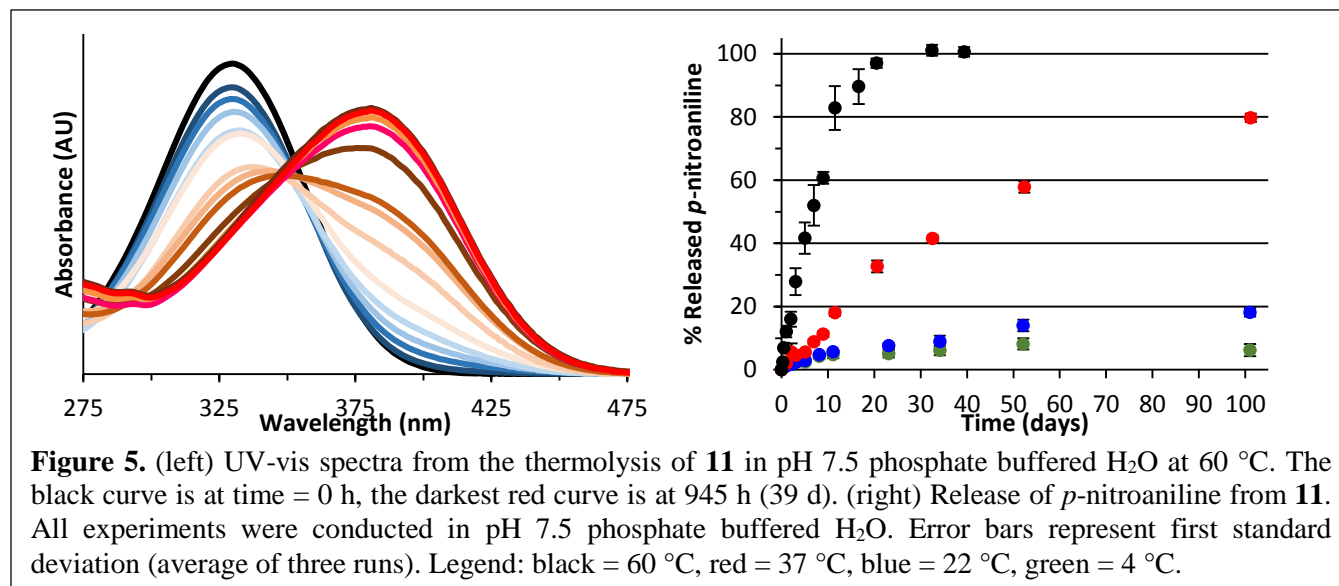
We found ring opening metathesis polymerization (ROMP) to be a successful strategy for norbornene-based monomers bearing oxazine moieties (Figure 4). Starting from known norbornene **7**, CDI coupling furnished the cyclopentadiene-functionalized norbornene **8**. Subsequent reaction with *N*-(*p*-nitrophenyl)hydroxy urea in the presence of Cu(I) salts provided the desired monomers (**9**) with oxazine adducts in 49% overall yield from **7**. Using a copolymerization with PEG-functionalized monomer **10** (1:3 molar ratio of **9** to **10**) provided a water soluble copolymer (**11**). Polymer **11** was analyzed by GPC and found to have  $M_n = 34.6$  kDa and  $\bar{D} = 1.04$ . By  $^1\text{H}$  NMR analysis, we calculated an average of 16 oxazine units per polymer chain.

The polymers were then dissolved in phosphate buffered H<sub>2</sub>O and the release of *p*-nitroaniline was monitored by UV-vis spectrometry at various temperatures (Figure 5). The oxazine adduct shows a  $\lambda_{\text{max}}$  near 329 nm, whereas the released *p*-nitroaniline shows a  $\lambda_{\text{max}}$  near 390 nm. A representative set of UV-vis spectra are shown in Figure 5 (left), and reveal a steady decrease in the amount of oxazine adduct with concomitant increase in the absorbance that



corresponds to *p*-nitroaniline. Confirmation of *p*-nitroaniline being released was further corroborated by  $^1\text{H}$  NMR spectroscopy. In Figure 5 (right) is shown the % of released *p*-nitroaniline over time at various temperatures. All samples were evaluated in pH 7.5 buffered water. At a cold storage temperature of 4 °C, we observed an expectedly slow release (green circles). Similarly, at 22 °C we observed less than 20% production of *p*-nitroaniline after 100 days. Increasing the temperature to 37 °C increased the rate of release considerably, and a steady kinetic profile was observed over the experiment time course of 100 days. This is exciting to us in that it signifies an ability for sustained release of HNO and small molecule organics (or SIP activation) at physiological

temperatures. As expected, increasing the temperature to 60 °C resulted in considerably faster breakdown of the adduct, which indicated to us that local heating techniques or photothermal transduction may be a viable method for augmenting the oxazine activation in these systems.



We have also investigated the mechanochemical potential of oxazines using computational methods (Figure 6). Density functional theory calculations at the B3LYP 6-31G\* level of theory using the Gaussian program package were used to simulate the effects of mechanical force on the oxazine model triggers. We used the Constrained Geometries Simulate External Force (CoGEF) method, which entails incremental distortions of the molecule along a specified coordinate. Relaxation after each distortion models the effects of mechanical force. Thus far, we have examined oxazines based upon cyclopentadiene and cyclohexadiene (Figure 6, top and bottom, respectively) and have observed interesting regiochemical implications for the mechanochemical reactivity.

For example, when attachment was modeled at the bridgehead of each oxazine class, clean cycloreversion reactions were observed. Although CoGEF results are not an absolute predictor of reactivity, they will be instrumental in guiding our initial synthetic outlay. Our future research will further correlate the CoGEF predictions with empirical results upon varying the site of polymer attachment on the mechanophore.

